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(54) **Title: MOLECULAR CLONING USING ROLLING CIRCLE AMPLIFICATION**
(54) **Titre: CLONAGE MOLECULAIRE A AMPLIFICATION SELON LE MODELE DU CERCLE ROULANT**

(57) Abstract

Disclosed are reagents and a method for efficient in vitro molecular cloning of nucleic acid molecules of interest. Because the method is entirely in vitro, it can be automated and scaled-up in ways that are not possible in cell-based molecular cloning. The method involves insertion of a nucleic acid molecule of interest in a linear vector to form a circular vector where one strand is continuous and the other strand is discontinuous. The continuous strand of the circular vector is then amplified by rolling circle replication, amplifying the inserted nucleic acid molecule in the process. The amplification is rapid and efficient since it involves a single, isothermic reaction that replicates the vector sequences exponentially. The amplification process is amenable to automation where multiple reactions are carried out simultaneously in a small area. The amplified nucleic acid can be used for any purpose and in any manner that nucleic acid cloned or amplified by known methods can be used. This includes sequencing, probing, restriction analysis, subcloning, transcription, hybridization or denaturation analysis, further amplified, and storage for future use or analysis.

(57) Abrégé

L'invention concerne des réactifs et un procédé permettant d'assurer un clonage moléculaire in vitro de molécules d'acides nucléiques déterminées. Etant entièrement in vitro, le procédé peut être automatisé et étendu selon des modalités qu'il est impossible de mettre en oeuvre avec le clonage moléculaire à base cellulaire. Le procédé consiste à insérer la molécule d'acide nucléique concernée dans un vecteur linéaire, de manière à former un vecteur circulaire dans lequel un brin est continu et l'autre brin est discontinu. Le brin continu est ensuite amplifié selon le modèle du cercle roulant, ce qui permet d'amplifier par la même occasion la molécule d'acide nucléique. L'amplification est rapide et efficace car elle fait intervenir une réaction isothermique unique qui assure la réplication exponentielle des séquences du vecteur. Le procédé peut être automatisé lorsque plusieurs réactions sont conduites simultanément dans une zone de taille réduite. L'acide nucléique amplifié peut être utilisé à toute fin et de façon quelconque dans l'éventail d'utilisation connu de l'acide nucléique cloné ou amplifié, y compris le séquençage, la détermination de sondes, l'analyse de restriction, le sous-clonage, la transcription, l'analyse d'hybridation ou de dénaturation, le complément d'amplification et le stockage pour utilisation ou analyse ultérieure.

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(57) Abstract

Disclosed are reagents and a method for efficient *in vitro* molecular cloning of nucleic acid molecules of interest. Because the method is entirely *in vitro*, it can be automated and scaled-up in ways that are not possible in cell-based molecular cloning. The method involves insertion of a nucleic acid molecule of interest in a linear vector to form a circular vector where one strand is continuous and the other strand is discontinuous. The continuous strand of the circular vector is then amplified by rolling circle replication, amplifying the inserted nucleic acid molecule in the process. The amplification is rapid and efficient since it involves a single, isothermic reaction that replicates the vector sequences exponentially. The amplification process is amenable to automation where multiple reactions are carried out simultaneously in a small area. The amplified nucleic acid can be used for any purpose and in any manner that nucleic acid cloned or amplified by known methods can be used. This includes sequencing, probing, restriction analysis, subcloning, transcription, hybridization or denaturation analysis, further amplified, and storage for future use or analysis.

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